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DATA EVALUATION REPORT II

STUDY TYPE: Metabolism - rat

MRID NO: 420075-02 TOX. CHEM. NO. 112701

TEST MATERIAL: Brodifacoum

SYNONYMS: Talon

STUDY NUMBER(S): URO172, URO211

SPONSOR: ICI Americas Inc.
Agricultural Products
Wilmington, DE 19897

TESTING FACILITY: ICI Central Toxicology Laboratory
Alderley Park, Macclesfield
Cheshire, UK

TITLE OF REPORT: Brodifacoum: Elimination from the Tissues of Rats Following Administration of Single Oral Doses.

AUTHOR(S): Batten, P. L.

REPORT DATED: December 5, 1990

CLASSIFICATION: Core supplementary data. This study does not satisfy the general metabolism data requirement (Guideline 85-1) for purposes of supporting the registration/reregistration of products consisting of or containing brodifacoum.

CONCLUSIONS:

1. Overall, the study is classified as core supplementary data. The study does not provide information as to how much brodifacoum was retained in carcasses of animals dosed with 0.02 or 0.35 mg/kg. There are insufficient analytical data relating to amount of label present in excrement, so it is not possible to correlate loss of label via the feces and/or urine with the half-life for elimination from the liver and other organs.

2. The major findings of this study involve the high retention and long-term persistence of the parent compound in the liver following a single oral dose of brodifacoum at both subtoxic (0.02 and 0.15 mg/kg) and toxic dose levels (0.35 mg/kg). We can accept the summary statement (p. 10) that: "The elimination of radioactivity from liver following administration of a toxic dose of brodifacoum was biphasic. There was a rapid phase which also corresponded to a reduction in clotting factor synthesis followed by a slower terminal phase during which blood clotting function was normal. The half life of elimination during the rapid phase (days 1-4) was approximately 4 days and for the slower phase (days 28-84) was 128 days. At non-toxic dose levels...the results showed that probably only the slow elimination phase was present for which the half-life was 350 days."

3. There is no indication that any attempt was made to determine whether or not the label present in urine and/or feces was present as metabolite(s) or parent compound, and what the proportions might have been.

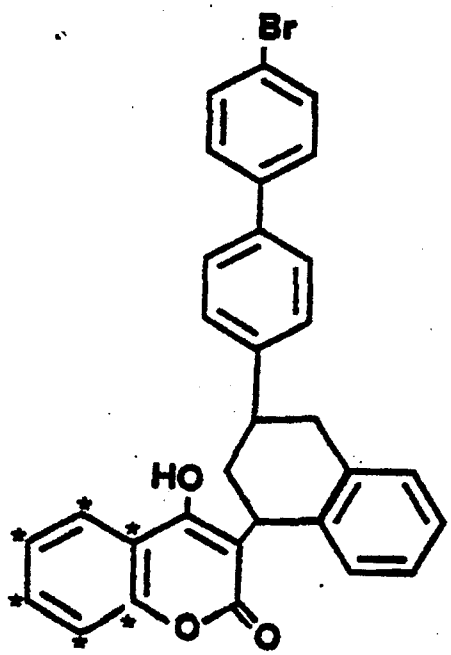
4. In addition, the way the study was conducted does not conform to the protocol recommendations in the Subdivision F Guidelines. The study did not include a group given a low-dose of non-labelled brodifacoum daily for 14 days, followed by a single non-toxic dose of labelled test material, nor did it include a group receiving an intravenous dosage of test material (although this deficiency, by itself, would not necessarily make the study unacceptable, considering that most of the oral dose was apparently absorbed).

A. MATERIALS:

1. Unlabelled test compound: Brodifacoum, test substance number 00052/018/003. The substance was 96% pure with a cis:trans ratio of 60:40.

2. Labelled test material: From p. 11: "Two samples of ^{14}C-brodifacoum, uniformly labelled in the phenyl ring of the coumarin moiety, were used. Test substance Y00052/010/004 was obtained from Imperial Chemical Industries PLC...with a specific activity of 546MBq/m mole. The radiochemical purity of the substance was determined by thin-layer chromatography...and found to be 96.1% and the cis:trans ratio was 59:41."

"Test substance Y00052/028/002 was obtained from Imperial Chemical Industries PLC...with a specific radioactivity of 1961MBq/m mole. The radiochemical purity was determined by thin-layer chromatography and found to be 95.3% and the cis:trans ratio was 61:39."



* Denotes position of ^{14}C atoms.

3. Test animals: From p. 14: "Adult male Alpk:AP rats (approximately 7 weeks old) were obtained from the Animal Breeding Unit, Alderley Park, Macclesfield, UK. The animals were then housed in stock rat cages...and provided with pelleted PCD rat diet...and water ad libitum. The animals were allowed to acclimatise for 7 days (Study No. URO172) and 5 days (Study No. URO211) prior to dosing."

B. STUDY DESIGN:

1. Dosage: From p. 13: Four groups of animals were dosed according to the following regimen:

Group	Study Number	Animal Numbers	Nominal Dose (mg/kg)	Nominal Dose (MBq/kg)
1	UR0172	1-21	0	0
2	UR0172	22-60	0.02	0.02
3	UR0172	61-120	0.15	0.15
4	UR0211	31-78	0.35	1.0

"A computer generated random number sequence was used to allocate these numbers." (presumably this means that the rats were assigned to the different dose groups using a random number sequence).

On p. 9 it is stated: "Two groups of male rats were given a single non-toxic dose of ^{14}C-brodifacoum (0.02 mg/kg or 0.15 mg/kg) and a further group was given a toxic dose (0.35 mg/kg). Animals were killed in groups of 3 at specific times for up to two years after dosing and blood and selected tissues taken for analysis." On page 11 it is stated that single oral doses of radio-labelled brodifacoum were administered "to male rats at dose levels of 0.02 mg/kg, 0.15 mg/kg and 0.35 mg/kg. The latter dose level was selected in order to elicit a toxic effect."

From p. 12: "The dose vehicle was polyethylene glycol (PEG400)..." From p. 14: "Animals in each group were given a dose of 5 ml dosing solution per kg of bodyweight (groups 1, 2 and 3) or 4 ml/kg (group 4)..."

2. Animal sacrifice: From information on pages 27, 29, and 31 rats were sacrificed at the following times after dosage:

Time After Dosing Dosing	Group 2 0.02 mg/kg	Group 3 0.15 mg/kg	Group 4 0.35 mg/kg
6 hrs	-	-	x
12 hrs	-	-	x
18 hrs	-	-	x
Day 1	x	x	x
Day 2	-	-	x
Day 3	-	-	x
Day 4	-	-	x
Day 8	-	-	-
Week 2	-	x	x
Week 4	x	x	x
Week 8	-	x	x
Week 12	-	-	x
Week 13	x	x	-
Week 26	-	x	-
Week 39	x	x	-
Week 52	-	x	-
Week 65	x	x	-
Week 78	-	x	-
Week 91	x	x	-
Week 104	x	x	-

x indicates 3 rats from the group were sacrificed at that time; - indicates no animals were sacrificed from the group at that time..

There is no indication within the report as to what (if anything) was done with the rats in group 1.

3. Preparation of samples for measurement of radioactivity: From p. 16: "Replicate weighed aliquots of dried faecal residues, blood, tissue samples or homogenates and carcass homogenates were combusted in a Packard Tricarb Model B306 sample oxidiser. ^{14}C-carbon dioxide was absorbed in OPTISORB '1' and automatically mixed with scintillant (OPTISORB 'S')... The efficiency of oxidation was determined by combusting known amounts of [^{14}C]brodifacoum and comparing the count after combustion with the count obtained from a blank sample which was combusted and then 'spiked' with the same amount of radioactivity. Results were corrected for combustion efficiencies which were determined at regular intervals during each series of sample combustions."

4. Data analysis: From p. 18: "Results are presented as mean values with standard deviations in the tables and as individual values in the appendices. Mean and individual data expressed as % values and as nanomole equivalents per g (nmol equiv/g) are presented to two decimal places. Apart from blood and fat, where only part of the tissue was taken the tissue residue data are presented additionally as % of the dose per tissue. All values presented...are accurate to the number of decimal places given. The limit of detection of radioactivity was taken as 46dpm (twice the liquid scintillation counter background rate)... The half-life of the radioactive species in liver was determined by linear regression analyses using a Commodore PET computer. The data were regressed to give a log 10/linear fit, a correlation coefficient and an equation of the line from which the half-life of elimination was calculated. The bodyweights of animals in groups 2 and 3 were compared with the control group using Student's t-test (two-sided)."

C. METHODS AND RESULTS:

1. Clinical observations, bodyweights, and mortalities: No information is given as to how frequently observations were made.

Results:

From p. 19: "A number of clinical observations were recorded during the study and for rats in groups 1 to 3, scabs, hairloss, piloerection and hunching were the most frequently recorded. None of the bodyweight changes or clinical observations were considered to be of biological significance for rats in groups 1 to 3 and therefore these data are not shown in this report. Those animals in group 4 which showed reduced bodyweight gain also exhibited subdued behaviour and hunching and had pale ears and tails. This was probably due to the effect of brodifacoum."

"None of the animals in groups 1 and 2 died during the first year of the study and only two deaths occurred with animals in group 3. During the second year a number of the animals in all three groups died but the numbers were not significantly different between the test and control groups and there was no evidence that any of the deaths were related to the administration of the test substance. None of the animals in groups 2 and 3 showed signs of internal haemorrhaging when dissected at the kill times. None of the animals in group 4 died but those which showed toxic effects...were killed... These and some of the other animals in the group showed signs of internal hemorrhaging when dissected. The surviving animals in all groups were killed at the end of the experimental phase of the study."

"Effects on bodyweight, mortalities and clinical observations could only be attributed to the administration of brodifacoum at the toxic dose level."

2. Coagulation times: At the time rats were sacrificed, "samples of blood (approximately 2 ml...) were taken by cardiac puncture and collected in tubes containing 0.11M trisodium citrate... The prothrombin time (PT) and kaolin-cephalin time (KCT) were measured..."

   Results:

   From p. 20: "At the two non-toxic dose levels the clotting times...were unaffected throughout the study and were within the normal range (approximately 14-24 sec for KCT and approximately 12-15 sec for PT) usually observed for rats in this Laboratory. The effect on coagulation was significant for rats given a toxic dose of brodifacoum. The prothrombin time reached a maximum of 148 seconds at 48h after dosing and was outside the normal range between 12 and 96h after dosing. After this time the values were within the range for normal animals."

   Refer to appended page 1 for the mean prothrombin and kaolin cephalin times.

3. Radioactivity in urine and feces: From p. 15: "Excreta were collected for the 24h period prior to the kill times... Excreta were not collected from group 4 animals. Three rats were transferred to individual metabolism cages designed for the separate collection of urine and faeces... The cages were washed with water at the collection time and the washings collected together with the urine. Urine and faeces were stored at -20° prior to radiochemical analysis."

Results:

The highest levels of radioactivity in the feces were observed in rats sacrificed at 24 hours post-dosing (group 2: a mean of 5.22% of the dose; group 3: 6.57%). The only measurable amount of radiolabel in the urine was observed in group 3 rats at 24 hours post-dosing. Refer to appended page 2 for mean levels of radiolabel (expressed as percentages of the administered dose).

4. Post-dosing concentrations of brodifacoum in different organs: The liver, kidneys, pancreas, salivary glands, and a 5 ml blood sample were taken at sacrifice, and the radioactivities of these samples were subsequently measured. In addition, for group 4 rats, a sample of abdominal fat was also taken. Measurements of the radioactivity of the frozen carcasses were also made on some occasions for group 3 animals.

Results: A considerable amount of the dose was retained in the liver, even at 104 weeks. From information on p. 28, group 2 males still retained a mean of 11.78% of the dose in their livers at week 104; for group 3 males it was 11.74%. for group 4 males it was 21.24% at week 12 (week 13 values for groups 2 and 3 were 34.01% and 31.74% respectively). Refer to appended pages 3, 4, and 5.

From p. 20: "For animals given non-toxic doses of brodifacoum (groups 2 and 3) the highest concentration of radioactivity was found in liver 1 day after dosing. Smaller and somewhat similar concentrations of radioactivity were present in pancreas, salivary glands and kidneys at that time. Subsequent elimination of radioactivity from the liver of both groups of rats occurred at a similarly slow rate throughout the 2 year period... The half-life of elimination of radioactivity (excluding the day 1 value) wa 350 days for both groups with a correlation coefficient of 0.96 for both values. The concentrations of radioactivity in salivary glands and kidney showed some fluctuation between day 1 and week 4 probably as a result of redistribution or metabolism and elimination of brodifacoum. The concentration of radioactivity in the pancreas of rats in both groups increased steadily up to week 13 after dosing by which time it was greater than the corresponding value for liver."

5. Liver analysis: From p. 16: "Aliquots (3-10g) of pooled liver homogenates from rats in group 3...and group 4...were analysed. The livers from animals in group 2 were not analysed. Group 3 samples were exhaustively extracted with chloroform and group 4 samples with a mixture of dichloromethane:acetone (50:50 v/v)(20-25 ml). After centrifugation

the extracts were separately bulked and analysed for radioactivity when recoveries ranged between 97 and 117% of the radioactivity in the liver. The extracts obtained from group 3 liver samples were further purified...followed by elution with chloroform and collection of 1 ml fractions. The applied radioactivity was quantitatively recovered in the first two fractions and these were reduced to low volume (0.25 ml) for high performance liquid chromatography. The extracts obtained from group 4 liver samples were reduced to low volume (0.5 ml) and chromatographed on thin layers without further processing."

Results: Refer to appended page 6. From p. 23: "A more polar component was present in the livers of group 4 rats which could not be detected in the livers of group 3 animals and accounted for 11% and 9% of the radioactivity in the day 1 and day 14 extracts respectively. Two additional minor components (<1%) were also found. These data showed that at either dose level and irrespective of the time after dosing brodifacoum was the major component found in the liver and the cis:trans isomer ratio of the substance was not significantly altered. The more polar component was shown to be present in liver in previous studies..."

D. DISCUSSION:

This is a very difficult study to review. For example, it is stated (p. 14) that: "the bodyweights of each rat in groups 1 and 3 were recorded weekly for the first 10 weeks and every 2 weeks for the remainder of the study. The bodyweights of rats in groups 2 to 4 were also recorded when killed for dissection." However, the only bodyweight data presented are in appendix 2 (pages 41-45), giving individual weights at the time of dosage. The statement is made on p. 19 that: "None of the bodyweight changes or clinical observations were considered to be of biological significance for rats in groups 1 to 3 and therefore these data are not shown in this report." It is also stated that: "Some of the animals given a toxic dose (0.35 mg/kg, group 4) showed reduced bodyweight gains at sacrifice when compared with other animals in the group." This reviewer's immediate interpretation is that group 4 animals did not have a concurrent control group (but if this is so, why were numbers 31-78 allocated to this group? Were numbers 1-30 assigned to concurrent controls?). On p. 19 it is stated: "None of the animals in group 4 died but those which showed toxic effects (rats 54, 61, 65, 67 and 69) were killed..." No information is presented as to when these rats were killed because of their symptoms, but animals 31-51 represented scheduled sacrifices in the period from 6-96 hrs after dosage. The next group of 3 rats was sacrificed on day 8, and included #52, #53, and #55, so #54 must have been killed between day 4 and day 8. However, no information is given as to when individual rats of this group

were killed (as a result of showing symptoms), with bodyweight data, gross pathological findings for individual animals, and/or clinical observations.

From the way the study was conducted, there are a number of uncertainties as to how much of the test material was actually retained by some of the animals and the rates at which it was excreted. The radioactivity in carcasses was only measured for group 3 (dosage: 0.15 mg/kg) animals, and not for group 4 (dosage: 0.35 mg/kg). However, in group 3 animals, most of the retained brodifacoum was in the carcass (week 52: a mean of 23.73% of the initial dose remained in the carcass, while a mean total of 46.5% - which includes that present in carcass - was retained by the animals). In short, since carcass radioactivity was not measured in groups 2 and 4, there is uncertainty as to how much total brodifacoum was retained in these groups. It is not possible to make estimates of the total body burden from the amounts of dose excreted in the urine and feces, since radioactivity was measured only for urine and fecal samples from groups 2 and 3 (not 4), and then only from collections from animals in the immediate 24-hour period before their sacrifice. Since, for group 3, the initial sacrifice of 3 animals was at day 1 (24 hours), and the next sacrifice was at week 2, this leaves a gap of 12 days (from 24 hours after dosage to 13 days) with no information.

No identification was made in this report as to the identity of the polar component (representing about 10% of the radioactivity present) in the liver of group 4 rats. However, considering the toxicity of the parent compound, this is probably a minor point (on a worst-case basis, the polar component could be considered to have the same toxicity as brodifacoum).

In addition, the way the study was conducted does not conform to the protocol recommendations in the Subdivision F Guidelines. The study did not include a group given a low-dose of non-labelled brodifacoum daily for 14 days, followed by a single non-toxic dose of labelled test material, nor did it include a group receiving an intravenous dosage of test material (although this deficiency would not necessarily make the study unacceptable, considering that most of the oral dose was apparently absorbed).

There is no indication that any attempt was made to determine whether or not the label present in urine and/or feces was present as metabolite(s) or parent compound, and what the proportions might have been.

The major findings of this study involve the high retention and long-term persistence of the parent compound in the liver following a single oral dose of brodifacoum at both subtoxic (0.02 and 0.15 mg/kg) and toxic dose levels (0.35 mg/kg). We can accept the summary statement (p. 10) that: "The elimination of radioactivity from liver following administration of a toxic dose of brodifacoum was biphasic. There was a rapid phase which also corresponded to a reduction in clotting factor synthesis followed by a slower terminal phase during which blood clotting function was normal. The half life of elimination during the rapid phase (days 1-4) was approximately 4 days and for the slower phase (days 28-84) was 128 days. At non-toxic dose levels...the results showed that probably only the slow elimination phase was present for which the half-life was 350 days."

The study also demonstrates that, at least for rats receiving the 0.15 mg/kg dose, a considerable amount of brodifacoum (slightly more than the total amount in the liver) was present in the carcass.

Overall, the study is classified as core supplementary data. The study does not provide information as to how much brodifacoum was retained in carcasses of animals dosed with 0.02 or 0.35 mg/kg. There are insufficient analytical data relating to amount of label present in excrement, so it is not possible to correlate loss of label via the feces and/or urine with the half-life for elimination from the liver.

Page ___ is not included in this copy.

Pages 11 through 16 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Inhalation LC_{50} Males = 4.86 µg/L (based on particulate concentration)
Females = 3.05 µg/L
Combined = not reported

Brodifacoum is in TOXICITY CATEGORY I (inhalation LC_{50} at or below 50 µg/L), based on LC_{50} values in both sexes.

Mortalities (accompanied by symptoms consistent with anti-coagulant activity) occurred on days 4, 5 and 6 in 3/5 males and 5/5 females exposed to the highest concentration.

This acute inhalation study is classified as acceptable. It <u>does</u> satisfy the guideline requirement for an acute inhalation study (81-3) in the rat for technical brodifacoum.